Review

Use of Saliva for Diagnosis of Diseases

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Abstract

Background and Aim: Timely diagnosis is an important factor in successful treatment of diseases. In. this regard, non-invasive sampling methods such as salivary analysis may be of great help in assessment of diseases and treatment outcomes.
Currently, introduction of new techniques for salivary analysis has increased its potential as a new diagnosing tool.
Materials and Methods: The National Institute of Dental and Craniofacial Research (NIDCR) was the first to propose the role of saliva in diagnosis and assessment of diseases. Since then, several studies have been conducted in this respect. This review article aims to evaluate the results of these studies to provide a brief insight on the correlation of saliva with different oral and systemic diseases. Key words namely saliva, biomarkers, oral diseases and systemic diseases were searched in PubMed, Google Scholar, EBSCO, Scopus and Medline databases and articles relevant to the use of saliva for evaluation and detection of various diseases published during 1950-2011 were collected.
Results: Salivary analysis increases the collaboration between dentists and physicians and can be used for detection of some systemic conditions.
Conclusion: This review study discusses the role of saliva in detection of different oral and systemic conditions and emphasizes its importance.

Key Words: Saliva, Biomarkers, Oral diseases, Systemic diseases

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Introduction

The main salivary glandsare in the form of pairs and include the parotid, the submandibular and the sublingual glands. These glands secrete saliva into the oral cavity. The secreted saliva is composed of a combination of serous and mucosal components. A total amount of 1-1.5L of non- stimulated saliva is produced daily by these glands. The share of each gland in secretion is different, with the submandibular glands having 65%, parotid 23% and sublingual glands having 4% of the share;the minor salivary glands secrete only a small amount (8%) of saliva [1]. In a more general sense, saliva can be divided into:
a) gland-specific saliva and
b) whole saliva [2]. Several factors affect the flowof secretion and the physiological characteristics of saliva. These factors include the circadian rhythm and physical activity [3]. Saliva is a clear, relatively acidic liquid (pH=6.0-7.0) containing electrolytes, immunoglobulins, proteins and different enzymes that play important roles in oral health. The primary roles of saliva include protecting the integrity of mucosal membrane, lubrication,

buffering and cleaning the oral cavity, antimicrobial effects, gustatory sense and primary digestion of food [1]. In the recent years, saliva has been extensively studied and the review of literatureshows that saliva can beused as a biological sample fordiagnosis of diseases. The most important advantage of saliva analysis is its ease of use, accessibility and non-invasiveness. Additionally, it is possible to look for hormonal, microbiological, immunological, pharmacological and oncological markers in the saliva and compare their salivary concentrationwith their plasma concentration [4]. Many blood markers enter the saliva through the intercellular space and trans-cellular pathways (passive transmission and active transmission) or through paracellular pathways (extracellular ultrafiltration). Thus, the saliva reflects the physiological status of the body as efficiently as the plasma. Since the salivary concentration of these markers is less than their blood concentration, use of saliva for clinical diagnoses may not be sufficiently accurate [1]. Of course, the advent of new sensitive technologies such as molecular diagnostic techniques and nanotechnology has resolved this problem and proteins and salivary peptides are measured usingdifferent biochemical techniques like liquid chromatography, gel electrophoresis, capillary electrophoresis, nuclear magnetic resonance, immunoassay and Lectin probe analysis and they are used as diagnostic biomarkers [5].

Materials and Methods

The National Institute of Dental and craniofacial Research (NIDCR) was the first to introduce the role of saliva fordiagnosis of diseases.Many previous studies have been conducted in this respect and the aim of this review article is to collect, classify and present a summary of the results of previousstudies in suchway that the reader can access brief and precise information onthe correlation ofdifferent oral and systematic diseases with the composition of saliva. For this reason, key words describedbelow were searchedin Google, EBSCO, Scopus, Medline and PubMeddatabasesand papers regarding the useof saliva for diagnosis of diseases published between 1950 and 2011 were collected. The searched key words included“saliva”, “oral diseases” (dental caries, periodontal disease, lichen planus, etc.) and “systemic diseases” (inherited, autoimmune, malignancies, etc.).

Dental Caries

Different researchers have tried to find a correlation between the composition of saliva and prevalence of dental caries. Recently, the salivatests are commonly used for measuring certain oligosaccharides associated with dental caries [5]. Certain proteome changes in the saliva indicate dental caries. For example, proteins with high amounts of proline (PRP1 and PRP3), histatin1 and statindecrease in high-caries patientswhile the level of these proteins ishighin caries-free individuals. Histatin S and statinare useful predictors for caries [5,6,7]. Some studies have shown an association between theincreasednumber of microorganisms like Streptococcus mutans and lactobacilli in the saliva and dental caries [7]. The modified Saliva Check SM and Saliva Check sIgA are used forrapid and precise diagnosis of dental caries in the elderly and have been proven to be significantly useful [8]. In another study, physiochemical features like the flowof secretion, pH, the buffering capacity, calcium content, who protein andantioxidant levelwere comparedin the saliva of childrenwith and without dental caries. The results showed that the secretion of saliva, pH and the buffering capacity of saliva in children with dental caries were slightly lower; whilethewhole protein and anti-oxidant level were significantly higher than those in children without caries. Thetotal calcium content of the saliva of children with caries was significantly lower than that in caries-free children [9].

Periodontal Disease

Existence of certain periopathogens in the saliva indicates that saliva is an appropriate means for theanalysisof bacterial risk factors using DNA-based enzymes.This method is very useful to assess the course of periodontal disease [6]. Many studies have been performed using this method and different kinds of salivary proteins have been detected in periodontal disease [5]. Comparison ofthe salivary proteome of patients with invasive periodontitis andhealthy subjects revealed that eleven salivary proteins such asalpha amylase, albumin and carbonic anhydrase 6 were different [5]. Previousstudies have measured markers like matrix metalloproteases(MMP), interleukins, hepatocellu-

lar growth factor (HGF), TNF-α and biomarkers related to bone turnover like the Macrophage Inflammatory Protein-1α (MIP-1α) and osteoprotegerinusing different methods and reported an increase in the amount of these biomarkers in periodontal disease [10,11,12,13,14,15]. In another study, salivary calcitonin in subjectswith type II diabetes reflected the severity of periodontitis and high blood sugar [16].A significant increase in salivary level of TLR2 and 4 (toll like receptor) was found in patients affected with periodontitis. These factorsenter the blood circulation from the gastrointestinal tract and increase the risk of atherosclerosis and insulin resistance [17]. Other studies have shown that high salivaryconcentration of PGE2 and TGF-β2 in HIV^+patientsis animportant risk factorforprogress of periodontitis [18, 19].

Oral Candidiasis

Saliva analysis is very efficient in diagnosis of oral mycosis. In oral candidiasis,fungi are colonized in the mucosa and make some changes in salivary proteomes. Proteins like calprotectin, histatin 5, mucin, peroxidase and high proline content proteins in the saliva have important diagnostic and prognostic rolesin this regard [6]. In onestudy, asignificant increase in salivary nitrates in these patientswas observed [20]. Also, a reduction in antimicrobial proteins in the saliva causes an increase in candidiasis [21]. In another ex vivo study, the protective role of histatin 5 in the saliva in protection of oral mucosa was introduced for the first time. The results of previous studies have shown a significant decrease in level of histatin 5 in HIV^+ positive patients with fungal colonization.

Lichen Planus

Salivary proteins are used as markers fordetection of oral diseases. For example in patients with lichen planus, a decrease in salivary protein PLUNG (palate, lung and nasal epithelium carcinoma associated protein) was observed [5]. In onestudy, the salivary concentration of IL10, STNFR-2, INF-α and INF-δ in patientswith erosive lichen planus was found to be high. The concentration of these markers decreased significantly after treatment with prednisone [24, 25]. In another study,level of IL-6, IL-1α and TNF-α reduced significantly after treatment with dexamethasone and the results showed that the measurementof cytokines was useful in monitoring the response to treatment.

Burning Mouth Syndrome (BMS)

Saliva hasalso been used for evaluation of BMS. Although this syndrome is idiopathic, neuropathy has been suggestedas a possibleetiology. Itmeans that there is an imbalance in substance P, Neurokinin A and calcitonin gene related peptide (CGRP) and the measurement of these neuropeptides in the saliva in comparison withserum showed that CGRP reduced in patientswith BMS. However, these findings were in contrast to theresults of Vanja et al, in 2010 [26, 27]. In another study, the amount of Nerve Growth Factor (NGF),substance P and products of degranulation of mast cells and neutrophils in the saliva wasmeasured in the control and BMS patients. The results showed a significant increase in NGF and mast cell products,a significant decrease in SP and no change in neutrophil markers. Thus, these factors can be used fordiagnosis and monitoring of BMS [29]. Also, in another study, a significant increase in salivary magnesium and no change in cytokines were notedafter measuring and comparing the concentration of cytokines and markers in the saliva of healthy controls and BMS patients [30].

Recurrent Aphthous Stomatitis and Recurrent Aphthous Ulcer

Aphthous is one the most common oral mucosal disorders with an unknown pathophysiology.Only a few factors are known to be responsible for its occurrence [31]. Researchers have measured the amounts of vitamins A, C and E and anti-oxidant MDA-5 (Malondialdehyde) in serum and saliva of patientswith RAU in comparison with healthy controls and concluded that the amount of these vitamins wassignificantly lower in patientscompared with healthycontrols while the amount ofMDA-5 was significantly higher in patients. Thus, there wasa significantcorrelation between the saliva and serum of these patients [32]. Another study showed a reductionin salivary EGF (epithelial growth factor) in patients with high frequency of RAU [33]. This marker has also been measured in patients with RAS and Behcet's disease and the results,similar tothe above-mentioned study,showed a decrease in the level of thisfactor. These findings indicate the role of EGF in pathogenesis of BD, RAS, RAU andmucosal disruption [34]. The results of another study showed a decrease in phagocytic function of salivary neutrophils similar to

blood neutrophils compared with healthy controls [35]. A similar study in this regard demonstarted that in the active phase of RAS, the salivary concentration of TNF-α is 2.5 times higher in patients than inhealthycontrols. Therefore, changes in TNF-α level have been suggested as the etiology of RAS [36]. Salivary and serum antioxidants have also been studied in patients with recurrent aphthous and the results have shown that changes in superoxide dismutase in red blood cells compared with other defensive substances like catalase and whole antioxidant playan important role in the inflammatory process seen in RAS [37]. The results of another study indicatedthat the concentration of salivary IgA in the active phases of RASwas significantly higher compared with the latent phases [38].

Systematic Diseases

Some systematic diseases affect the salivary glands either directly or indirectly and thus, change the components of the secreted saliva. Therefore, it is possible to use such changes forearly diagnosis, determination of severity and even prognosis of diseases.

Hereditary Diseases Cystic Fibrosis

Cystic fibrosis is a genetic disorder that results from a mutation in the GFTR gene that is located on the long armof chromosome 7 and mostly affects children and young adults. These people have general exocrinopathy and its recessive form is fatal. The abnormal secretion of exocrine glands in CF has led the clinicians to think about the efficacy of using saliva in diagnosis of this disease [39]. Increase in calcium, phosphorus, sodium, chloride, urea and uric acid in the saliva of these patients hasbeen reported. The abnormal forms of Epithelial Growth Factor (EGF) and PGE-2 arealso elevated in the saliva of these people. In addition, the activity of cathepsin D in these patientsis significantly higher than inhealthycontrols. Salivary thiocyanate in these patients is also higher than in normal controls;although more studies are needed in this regard [6, 39, 40, 41, 42].

Celiac Disease

Celiac disease is a gastrointestinal disorder related to permanent glutenintolerance that causes the destruction of intestinal mucosa through immunologic mechanisms in people who have genetic predisposition. Atrophy of intestinal villi causes malabsorption of different types of nutrients like calcium, iron and folate. The results of several studies indicate that the amount of IgA Antigliadin Antibody (IgA-AGA) is elevated in the serum of these patients and this increase is in perfect alignment with salivary IgA-AGA. Therefore, it is possible to use this factor with a good sensitivity and specificity forscreening of these patients. Some studies have even suggested the use of this test before performing intestinal villibiopsy [6, 39, 43,44]. Other researchers have measured the anti-tissue transglutaminase immunoglobulin A(tTG-Abs) in the serum and saliva of patients and have concluded that screening and diagnosis of this disease using this factor in the saliva has adequatesensitivity and is simple.Moreover,the salivary tTG-Abs is detectable in these patients even when on a non- gluten diet [45, 46]. Another study on this topic has reported an increase in peroxidase, myeloperoxidase, albumin, total protein, IgA and IgG and a decrease in IgM and amylase in the saliva of these patients [47].

Deficiency or Lack of 21-Hydroxylase

Deficiency or lack of 21-hydroxylase is a hereditary steroidogenesisdisorder that causes hyperplasia of the adrenal glands [48]. In the non-classic 21 hydroxylase disorder, there is partial deficiency of this enzyme. Primary monitoring of 17-dehydroxy progesterone of the saliva (17-DHP) is a perfect screening test for this disease because the salivary 17DHP reflects its concentration in serum [49, 50].

Sjögern'sSyndrome (an autoimmune disorder)

Sjögern's syndrome (SS) is an autoimmune disorder that mainly affects functions of lacrimation and salivary glands and causesserum abnormalities. Itcan be detected by expensive and invasive tests. Recently, the use of saliva has been discussed in many studies for the diagnosis of this disease; whichis quite valuableconsideringits non-invasiveness and affordable cost. Sialochemistry has been useful in diagnosis of Sjögern's syndrome. The results of saliva analysis in people with this disorder have shown an increase in inflammatory proteins, β-microglobulin, lactoferrin, lysozymeC, sodium, chloride, IgA, IgG, albumin and cystatinsand decrease in high proline containing proteins and amylase. Also, there is a reduction in the saliva flow in SS; which is not a pathognomonic feature of this syndrome but will cause oral symptoms like caries,

fungal infections, dysphagia and oral pains [39, 51, 52]. Genomic biomarkers are also present in the saliva of these patients. In onestudy, 25 sequences of amino acids were identified to beup-regulated and 16 sequences were found to bedown-regulated. Of these sequences, 10 up-regulated regions and 6 down-regulated regions were significantly useful fordiagnosis of SS. Also, 162 types of mRNA were identified in the saliva that lead tointerferon production, lymphocyte sequestration and antibody production involved in pathogenesis of SS [6]. In another study, the level of IL-7 in the saliva of minor glands in patientswith SS waselevated. This increasecancause absorption and differentiation of T cells and lead to secretion of different cytokines like monokines, IL-4, IL-17, TNF-α and IL-1α in the saliva of patients compared with healthy controls. Also, anincrease in level ofIL-18 (which is a pro-inflammatory and immunoregulatory cytokine and has a pathogenic role in autoimmune disease with Th1 involvement)was observed in serum and saliva of these patients that can indicate the involvement of this cytokine in the immunologic and inflammatory course of this disease. Proteomic analysis has also been used for distinction of primary and secondary Sjögern's syndrome [53, 54, 55, 56].

Malignancies

Early detection and treatment of cancers is important in increasinglife expectancy. Certain markers in the serum, saliva and other body fluids can be useful in detection of cancers. Thus, somestudies have used proteomic and genomic analyses for diagnosis and prognosis of cancers. Evidence shows thatmutation of P53 gene is detectable in the salivary DNA of 62.5% of patients with different oral cancers. Also, HPV virus DNA increases in head and neck cancers. Studies related to salivary mRNA profiling show4major biomarkers in the saliva of patients with oral cancer [ILI-β, IL-8, ornithine decarboxylase antizyme-1 (OAZ1) and spermidine N1 acetyltransferase (SAT)]. Salivary proteome has also shown an increase in defensin-1 and cancerous antigens CA 15-3, CA-125 and P53 in different cancers [6, 39, 57, 59, 58]. Increased levels of IL-6 and FGF-β have been found in the saliva and serum of oral SCC patients. Suchincrease probably indicates that the origin of these factors is the carcinomatous cells. Another study measured the amount of IL-1, IL-6, IL-8 and TNF-α in patients with oral SCC as well as patients with pre-cancerous disease in comparison with healthy controls. The results showeddifferent levels of these cytokines in OSCC and OPML;therefore, these pro-angiogenic and pro-inflammatory cytokines can be used for diagnostic and prognostic purposes [60, 61, 62]. In another study the amount of salivary endothelin-1 in oral SCC and leukoplakia was studied and the results indicated that the amount of this factor waselevated in OSCC; but it wasnot a good maker forevaluationof the degree of malignancy [64]. Other studies detected asignificant increase indifferent makers like IL-6 and IL-8 and also salivary glutathione of patients with oropharyngeal SCC compared with healthy controls [65, 66]. In one study, a significant increase in the concentration of different MMP like MMP-7, MMP-9 and MMP-26 was detected in patients with SCC of the lower lip and tongue. Thus, MMP can be used as a diagnostic biomarker in this cancer [67]. In another study, the amount of magnesium, calcium, copper, chloride, potassium, sodium, total protein and amylase was measured and compared between OSCC patients and healthycontrols. The results showed higher levels ofsalivary sodium and chloride and lower level of salivary total protein in these patients compared withcontrols [68]. Moreover,a similar study examined changes in8 salivary biomarkers in patients with cancer and reported an increase in5 markers (MMP, Ki 67, cyclin D1, lactate dehydrogenase and carbonyl) and a decrease in3 makers (Maspin, phosphorylated-src and 8-oxoguanine-DNA). Since these markers have high sensitivity and specificity, they can be very usefulin diagnosis of cancers in other parts of the body. For example, prostate specific antigen (PSA) in thesaliva that is in complete correlation with blood PSA is useful for diagnosis of prostate adenocarcinoma [70]. Additionally, transcriptomic biomarkers in thesaliva with high specificity and sensitivity have a good application in diagnosis of pancreatic cancer [71]. On the same topic, a study examined the transcriptomic and proteomic biomarkers in patients with breast cancer and healthy controls not influenced with confounding factors. The results revealed that these markers hadhigh sensitivity and specificity fordiagnosis of breast cancer [72]. In other studies, there was a significant increase in

factors like EGF, C-erb β2, CA-125, glycoprotein and CA 15-3 in the saliva of patientswith breast and ovarian cancers [58, 59, 73, 74, 75, 76, 77].

Endocrinology

Collection of saliva facilitates determination of hormonal level and diurnal variation [78]. Studies have shown that salivary cortisol is a valuable indicator of serum cortisol which is not dependent on salivary flow. Indeed,clinical assessment of most endocrine glands requires monitoring of plasma steroids. It has been found that while the amount of steroid hormones in the saliva reflects the free and active amounts of thesehormones, the blood shows its total amount (free and bound) [76]. Different studies haveevaluatedsalivary levels of cortisol, estradiol, progesterone and testosterone. Since these hormones play animportant role in mental healthof patients, they can also be used in the field of psychology. It has been shown that testosterone level in women with breast cancer is significantly low [79]. In another study, the salivary levels of progesterone and estriol were assessed in pregnant women and the increase of estriol was found to increase the risk of premature labor [80, 81]. The amount of salivary cortisol in other studies indicated the level of serum cortisol. Based on the literature, the morning concentration of salivary cortisol can be used for the diagnosis of Addison's disease and its nocturnal concentration can be used fordiagnosis of Cushing's disease [82, 83, 84, 85]. Another study assessing the seasonal changes of salivary testosterone concentration concluded that the concentration of this hormone increased in autumn and decreased in summer. These results can now be used for research in the field ofpsychology and endocrinology [86]. Exercise also changes the salivary concentration of steroid hormones, immunoglobulins, antimicrobial proteins and enzymes. The levelof these hormonesincreases in irregular and unprofessional activities and decreases in professional sportsmen [87]. Likewise, it is possible to use cortisol concentration in non-stimulated saliva early in the morning for screening of patients predisposed to secondary adrenal insufficiency [88]. Beside these applications, the saliva can also be used for measurement of aldosterone [89, 90].

Diabetes

Different studies have shown that treatment-resistant periodontitis can be an indicatorofundiagnosed or uncontrolledtype 2diabetes. Thus,resistant periodontal disease is a risk factor forcardiometabolic diseases likediabetes [78]. On the other hand, certain salivary biomarkers are helpful in diagnosis of type 2diabetes [91]. One study showed a significant increase in the activity of proteases like cathepsin D and MMP-9 in healthy controls and people with diabetes in such way that collagen fragmentscan be used for monitoring of pathologies related to diabetes [92]. The results of another study showed a significant increase in salivary IL-6 in diabetic patients who also hadlichen planus compared with other groups (healthy controls, diabetics and people with lichen planus) [93]. In another study, use of saliva containing immunologic factors in people with diabetes led to a reduction in plaque, gingivitis and fungal diseases [94]. The analysis of saliva in another study showed an increase in salivary glucose and a decrease in salivary amylase of diabetic patients (controlled and uncontrolled) [95].

Renal and Cardiovascular Diseases

Saliva has also been used for detection of renal diseases. Some studies measured the salivary creatinine level andpredicted the renal disease with high sensitivity and specificity [75].

B2-microglobulin is a major component in dialysis-related amyloidosis. In one study the amount of this marker was evaluated in the saliva and serum of healthysubjects, diabetics, patients with chronic renal disease and subjectsunder hemodialysis. The resultsshowed that analysis of salivary β2-microglobulin wasa reliable method forassessing its serum concentration and can be used to predict the risk of amyloidosis, secondary to chronic renal disease [96]. Hyperphosphatemia is an important factor in vascular calcification in people with chronic renal failure. This calcification is the main cause of mortality in patientsunder hemodialysis and needs to be treated. The salivary phosphate after being swallowed also contributes to this problem. Since salivary phosphate is in perfect accordance with serum phosphate, it can be a good marker forinitiationof treatment in this process [97]. Another study used salivary urea and nitrogen whichare inperfect accordance with blood urea and nitrogen for detection of chronic renal failure [98]. The results of a similar study indicated that the salivary concentration of creatinine, urea, sodium,

potassium, chloride and α-amylase in patients with chronic renal failure was higher than in the control group whilethe calcium level was significantly lower. It should be noted that the components of saliva varywith the severity of disease [99]. Other studies have shown a significant decrease in salivary concentration of inflammatory cytokines in patientswith chronic renal failure [100]. Moreover, saliva can be used for monitoring of cardiovascular diseases. Currently, cardiovascular diseases are a major cause of mortality. Use ofsalivary markers formonitoring of these patientshas been confirmed especially after a cardiac surgery. An example of these markers is the salivary α-amylase; which is elevatedin stressful situations [6, 76].

Also,periodontal diseaseby increasingIL-6, TNF-α and C- reactive protein (CRP) playsan important role in risk determination of cardio-metabolic diseases. Biomarkers like myoglobin, myeloperoxidase and CRP have a significant diagnostic ability in myocardial infarction [78] and the amount of collagenases like MMP increases in cardiovascular diseases [13].

Other applications: Many studies have been performed on the efficacy of saliva in diagnosis of different viral and bacterial diseases and monitoring of different medications;which will not be discussed here due to the limitations of the current study.

Currently, there are many studies that have assessed different biomarkers (genomic, transcryptomic and proteomic) and found their correlation with somediseases. Specific kits are being designed for measuring salivary markers indifferent diseases. By doing so, a fast, cost effective and easy method willreplace the existingones. Using this test we can measure diagnostic markers; which willhelp in early detection of different diseases.

Researchers have recently introduced a kit for measuring CRP; which is a general indicator of inflammatory diseases [101].

It appears that with recent advancements in technology, saliva analysis will soon bea valuable method for diagnosis.

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